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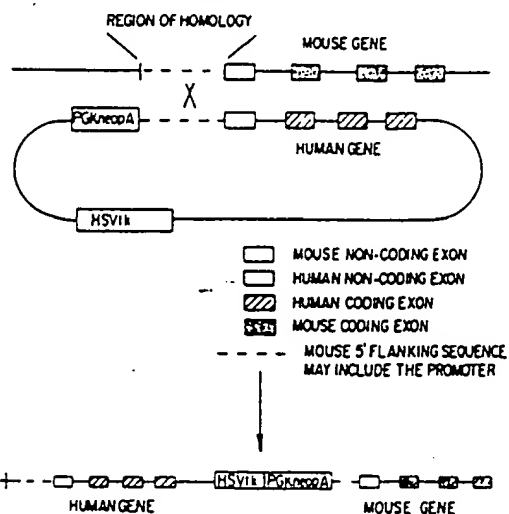
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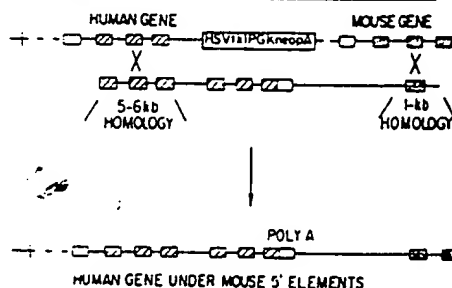
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(54) Title: METHOD FOR HOMOLOGOUS RECOMBINATION IN ANIMAL AND PLANT CELLS

STEP #1. HOMOLOGOUS RECOMBINATION: ADDING THE HUMAN REPLACEMENT WITH AN INSERTION VECTOR.



STEP #2. RECONSTRUCT JUNCTION. REMOVE DUPLICATED PROMOTER. ADD ADDITIONAL 3' HUMAN SEQUENCES. SELECT IN F1AU (100%)



(57) Abstract

A method for producing animal cells which contain a desired gene sequence which has been inserted into a predetermined gene sequence by homologous recombination. The method permits the production of animal cells which have subtle and precise modifications of gene sequence and expression.

WHAT IS CLAIMED IS:

1. A method for obtaining a desired animal or non-fungal plant cell which contains a desired non-selectable gene sequence inserted within a predetermined gene sequence of said cell's genome, which method comprises:

A. incubating a precursor cell with a DNA molecule containing said desired non-selectable gene sequence, wherein said DNA molecule additionally contains two regions of homology which flank said desired gene sequence, and which are sufficient to permit said desired gene sequence to undergo homologous recombination with said predetermined gene sequence of said genome of said precursor cell;

B. causing said DNA molecule to be introduced into said precursor cell;

C. permitting said introduced DNA molecule to undergo homologous recombination with said predetermined gene sequence of said genome of said precursor cell to thereby produce said desired cell wherein said desired non-selectable gene sequence has been inserted into said predetermined gene sequence; and

D. recovering said desired cell.

2. The method of claim 1 wherein said DNA molecule contains a detectable marker gene sequence.

3. The method of claim 1 wherein said DNA molecule is introduced into said precursor cell by subjecting said precursor cell and said DNA molecule to electroporation.

4. The method of claim 3 wherein in step B, said precursor cell is simultaneously subjected to

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1 electroporation with a second DNA molecule, said second DNA
2 molecule containing a detectable marker gene sequence.

3
4 5. The method of claim 1 wherein said desired cell is a
5 non-fungal plant cell.

6
7 6. The method of claim 1 wherein said desired cell is an
8 animal cell.

9
10 7. The method of claim 6 wherein said animal cell is a
11 somatic cell.

12
13 8. The method of claim 7 wherein said animal cell is of
14 an animal selected from the group consisting of a chicken,
15 a mouse, a rat, a hamster, a rabbit, a sheep, a goat, a
16 fish, a pig, a cow or bull, a non-human primate and a human.

17
18 9. The method of claim 6 wherein said animal cell is a
19 pluripotent cell.

20
21 10. The method of claim 9 wherein said animal cell is of
22 an animal selected from the group consisting of a chicken,
23 a mouse, a rat, a hamster, a rabbit, a sheep, a goat, a
24 fish, a pig, a cow or bull, and a non-human primate.

25
26 11. The method of claim 9 wherein said pluripotent cell
27 is an embryonic stem cell.
28

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12. The method of any one of claims 1-3 wherein said desired gene sequence is substantially homologous to said predetermined gene sequence of said precursor cell.

13. The method of claim 12 wherein said desired gene sequence is an analog of said predetermined sequence of said precursor cell.

14. The method of claim 12 wherein said desired gene sequence is a human analog of said predetermined sequence of said precursor cell.

15. The method of claim 12 wherein said desired cell is a non-human cell which expresses said desired gene sequence.

16. The method of claim 12 wherein said desired gene sequence encodes a protein selected from the group consisting of: a hormone, an immunoglobulin, a receptor molecule, a ligand of a receptor molecule, and an enzyme.

17. A non-fungal plant cell which contains an introduced recombinant DNA molecule containing a desired gene sequence, said desired gene sequence being flanked by regions of homology which are sufficient to permit said desired gene sequence to undergo homologous recombination with a predetermined gene sequence of the genome of said cell.

18. A non-human animal cell which contains an introduced recombinant DNA molecule containing a desired gene sequence, said desired gene sequence being flanked by regions of homology which are sufficient to permit said desired gene sequence to undergo homologous recombination with a predetermined gene sequence of the genome of said cell.

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19. The desired cell produced by the methods of any one of claims 1-3.

20. The desired cell produced by the method of claim 11.

21. The desired cell produced by the method of claim 12.

22. A non-human animal containing a cell derived from the desired cell of claim 19, wherein said animal is either a chimeric or a transgenic animal.

23. The non-human animal of claim 22, wherein said animal and said desired cell are of the same species, and wherein said species is selected from the group consisting of: a chicken, a mouse, a rat, a hamster, a rabbit, a sheep, a goat, a fish, a pig, a cow or bull, and a non-human primate.

24. A non-human animal containing a cell derived from the desired cell of claim 20, wherein said animal is either a chimeric or a transgenic animal.

25. The non-human animal of claim 24, wherein said animal and said desired cell are of the same species, and wherein said species is selected from the group consisting of: a chicken, a mouse, a rat, a hamster, a rabbit, a sheep, a goat, a fish, a pig, a cow or bull, and a non-human primate.

26. A non-human animal containing a cell derived from the desired cell of claim 21, or a descendant thereof, wherein said animal is either a chimeric or a transgenic animal.

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1 27. The non-human animal of claim 26, wherein said animal
2 and said desired cell are of the same species, and wherein
3 said species is selected from the group consisting of: a
4 chicken, a mouse, a rat, a hamster, a rabbit, a sheep, a
5 goat, a fish, a pig, a cow or bull, and a non-human primate.
6

7 28. A non-fungal plant containing a cell derived from the
8 desired cell of claim 5, or a descendant thereof, wherein
9 said non-fungal plant is either a chimeric or a transgenic
10 plant.
11

12 29. A method of gene therapy which comprises introducing
13 to a recipient in need of such therapy, a desired non-
14 selectable gene sequence, said method comprising:
15

16 A. providing to said recipient an effective amount
17 of a DNA molecule containing said desired non-selectable
18 gene sequence, wherein said DNA molecule additionally
19 contains two regions of homology which flank said desired
20 gene sequence, and which are sufficient to permit said
21 desired gene sequence to undergo homologous recombination
22 with a predetermined gene sequence present in a precursor
23 cell of said recipient;

24 B. permitting said DNA molecule to be introduced
25 into said precursor cell;

26 C. permitting said introduced DNA molecule to
27 undergo homologous recombination with said predetermined
28 gene sequence of said genome of said precursor cell to
29 thereby produce a desired cell wherein said desired non-
30 selectable gene sequence has been inserted into said
31 predetermined gene sequence; and wherein the presence or
32 expression of said introduced gene sequence in said cell of
33 said recipient comprises said gene therapy.

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1 30. The method of claim 29 wherein said recipient is a
2 non-fungal plant.

3 31. The method of claim 29 wherein said recipient is an
4 animal.
5

6 32. The method of claim 31 wherein said animal is
7 selected from the group consisting of: a chicken, a mouse,
8 a rat, a hamster, a rabbit, a sheep, a goat, a fish, a pig,
9 a cow or bull, a non-human primate and a human.
0

1 33. The method of claim 32, wherein said animal is a
2 human.
3

4 34. A method for obtaining a desired animal or non-fungal
5 plant cell which contains a desired non-selectable gene
6 sequence inserted within a predetermined gene sequence of
7 said cell's genome, which method comprises:
8

9 A. incubating a precursor cell under non-selective
0 culture conditions, or under a first set of selective
1 culture conditions, with a DNA molecule containing:

- 2 i) said desired non-selectable gene sequence,
3 wherein said DNA molecule additionally contains
4 two regions of homology which flank said desired
5 gene sequence, and which are sufficient to permit
6 said desired gene sequence to undergo homologous
7 recombination with said predetermined gene
8 sequence of said genome of said precursor cell;
9 and
0 ii) a selectable gene sequence whose presence or
1 expression in said precursor cell can be selected
2 for by culturing said cell under said first set
3 of selective culture conditions, and whose
4

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1 presence or expression in said precursor cell can
2 be selected against by culturing said cell under
3 a second set of selective culture conditions;

4 B. permitting said DNA molecule to be introduced
5 into said precursor cell;

6 C. permitting said introduced DNA molecule to
7 undergo homologous recombination with said predetermined
8 gene sequence of said genome of said precursor cell to
9 thereby produce said desired cell wherein said desired non-
10 selectable gene sequence has been inserted into said
11 predetermined gene sequence; and

12 D. recovering said desired cell by culturing said
13 cell under said first set of selective culture conditions,
14 by then permitting said cell to undergo intrachromosomal
15 recombination under non-selective culture conditions, and by
16 then incubating said cell under said second set of selective
17 culture conditions.
18

19 35. The method of claim 34, wherein said cell is
20 deficient in HPRT enzyme, and wherein said selectable gene
21 sequence expresses an active HPRT enzyme, and wherein said
22 first set of selective culture conditions comprises
23 incubation of said cell under conditions in which the
24 presence of an active HPRT enzyme in said cell is required
25 for growth, and wherein said second set of selective culture
26 conditions comprises incubation of said cell under
27 conditions in which the absence of an active HPRT enzyme in
28 said cell is required for growth.
29

30 36. The method of claim 34, wherein said cell is
31 deficient in APRT enzyme, and wherein said selectable gene
32 sequence expresses an active APRT enzyme, and wherein said
33 first set of selective culture conditions comprises